

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

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Claims 1-20 have been amended as follows:

1. (Currently Amended) A method for identifying essential and non-essential genes target nucleotide sequences in a haploid genome of a cell grown in non-selective conditions, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target regions insertionally mutated is obtained;

growing said population of cells under non-selective conditions to provide a non-selected sub-population of cells;

amplifying a target region from said non-selected sub-population of cells, using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer

pair enabling the amplification of a second extension product; and

assessing for the presence or absence of said first and second extension product, whereby the presence of the first and second extension products is indicative of a non-essential ~~gene~~ target nucleotide sequence, whereas the presence of the first extension product and the absence of the second extension product is indicative of an essential ~~gene~~ target nucleotide sequence.

2. (Currently Amended) A method according to claim 1, wherein ~~mutagenizing saturation~~ mutagenesis is performed with a transposable element.
3. (Currently Amended) A method according to claim 2, wherein said target ~~DNA~~ nucleotide sequence comprises a gene encoding a protein.
4. Canceled.
5. (Currently Amended) A method according to ~~claim 4~~ claim 21, wherein ~~mutagenizing saturation mutagenesis~~ comprises the steps of:

combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising:

- (a) a recognition sequence for said retroviral integrase; and
- (b) a sequence tag,

wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules.

6. (Currently Amended) A method according to ~~claim 4~~ claim 21, wherein ~~mutagenizing saturation mutagenesis~~ comprises the steps of:

combining DNA comprising said target region with retroviral integrase and a first set of complementary oligonucleotide primers, said primers comprising:

- (a) a recognition sequence for said retroviral integrase; and
- (b) a recognition site for a type II restriction endonuclease,

wherein said retroviral integrase mediates the insertion of said first set of complementary oligonucleotide primers to provide a population of mutagenized DNA molecules cutting said population of mutagenized DNA molecules with said type II restriction endonuclease to provide cut DNA; and ligating to said cut DNA a second set of complementary oligonucleotide primers comprising a sequence tag.

7. (Currently Amended) A method according to claim 5, wherein said target nucleotide sequence of interest comprises a gene encoding a protein.

8. (Currently Amended) A method according to ~~claim 4~~ claim 21, wherein said selective condition is growth of cells in media lacking a nutrient that is an intermediate in a metabolic pathway.

9. (Currently Amended) A method according to claim 8, wherein said ~~population of mutagenized DNA molecules~~ target nucleotide sequences insertionally mutated are cloned into a filamentous bacteriophage vector with regulatory sequences for expression of said target nucleotide sequence of interest.

10. (Currently Amended) A method according to claim 5, wherein said target nucleotide sequence of interest comprises a regulatory gene.

11. (Currently amended) A method according to claim 10, wherein said selective condition is growth in media containing a cytotoxic agent, and wherein said regulatory gene controls expression of a gene conferring resistance to said cytotoxic agent.

12-13. Canceled.

14. (Currently Amended) A method according to claim ~~13~~ 1, wherein said haploid genome is a bacterial genome.

15-18 . Canceled.

19. (Original) A method according to claim 1, wherein said amplification is carried out by the polymerase chain reaction.

20. Canceled.

21. (New) A method for identifying essential and non-essential target nucleotide sequences in an haploid genome, said method comprising:

saturation mutagenesis of said genome by insertion mutagenesis, whereby an oligonucleotide sequence is inserted in the target regions of said genome such that a population of cells having at least 90% of said target nucleotide sequences insertionally mutated is obtained;

subjecting a first aliquot of said host cells to at least one selective condition and a second aliquot to a non-selective condition thereby providing at least one selected and one non-selected aliquot;

amplifying a target nucleotide sequence from said at least one selected and one non-selected aliquot using a first primer which hybridizes to a known first end of said target region, and a second primer which hybridizes to another known end of said target region, said first and second primers thereby constituting a first primer pair, giving rise to a first extension product, and a third primer which hybridizes to said oligonucleotide sequence, said third primer constituting a second primer pair with one said first or second primer, said second primer pair enabling the amplification of a second extension product; and

assessing for the presence, absence or difference in quantity of said first and second extension products whereby differences between the presence or intensity of amplified DNA bands between said at least one selected and one non-selected aliquots are indicative that said target nucleotide sequence causes a difference in response to said selective condition thereby determining if said target nucleotide sequence is essential or non essential under said selective conditions.

22. (New) A method according to claim 21, wherein said haploid genome is a bacterial genome.